Alternaria Leaf Spots of *Brassaia actinophylla, Dizygotheca elegantissima,* and *Tupidanthus calyptratus*

J. Y. UCHIDA, Research Associate, M. ARAGAKI, Plant Pathologist, Department of Plant Pathology, University of Hawaii, Honolulu 96822; and M. A. YOSHIMURA, Associate Professor, Department of Biological Science, California Polytechnic State University, San Luis Obispo 93407

ABSTRACT

Uchida, J. Y., Aragaki, M., and Yoshimura, M. A. 1984. Alternaria leaf spots of *Brassaia actinophylla, Dizygotheca elegantissima,* and *Tupidanthus calyptratus*. Plant Disease 68:447-449.

A severe leaf spot on *Brassaia actinophylla, Dizygotheca elegantissima,* and *Tupidanthus calyptratus* was shown to be caused by *Alternaria panax*. No evidence for host specialization was seen in cross-inoculations. Conidia from *Brassaia* leaf tissue were considerably larger than those formed on agar medium. Between 16 and 28 C, the longest conidia were produced at 20 C, whereas the widest were formed at 28 C. To a limited extent and for a limited time, conidia continued to grow on either substrate, developing into grotesque shapes suggestive of hyperplasia.

Additional key words: *Alternaria actinophylla, A. araliae, A. raphani,* Araliaceae, conidial morphology, *Schefflera actinophylla, S. arboricola*

An *Alternaria* sp. causing leaf spots on *Dizygotheca elegantissima* Vig. & Guill. and *Tupidanthus calyptratus* Hook. f. & Thoms. was recently identified as *A. panax* Whetzel (11). In Hawaii (12), this fungus was also shown to cause leaf spots on potted plants of *Brassaia actinophylla* Endl. (=*Schefflera actinophylla* (Endl.) Harms) (octopus tree). Typical symptoms on *Brassaia* and *Dizygotheca* were tan to dark brown leaf spots and rapid blighting of foliage under moist conditions. Chlorotic zones around necrotic lesions were common on both hosts, and considerable leaf drop was associated with the disease. On older leaves, lesions were smaller and had a raised corky border. On *Tupidanthus*, leaves were similarly spotted and young leaves were blighted. On all three hosts, numerous conidia of a large-spored *Alternaria* were found on blighted leaves.

During preliminary laboratory studies, a wide range of conidial size and shape was observed in all isolates. Conidia produced in agar culture were larger than those reported by Atilano (3) but failed to approach the reported lengths (150–160 μm) of *A. panax* spores described by Simmons (11) from host tissue or the upper half of the range (70–185 μm) reported by Miller (9) from unspecified substrates. We also observed that slender, longitudinally symmetrical conidia in young colonies appeared to change in old colonies into broad, asymmetric, severely distorted conidia resembling *A. chlamydospora* Mouchacca.

Although these variations have been noted on host tissue (11), a detailed mycological study establishing the range of morphological changes in conidial development is lacking. The objectives of this study were to determine pathogenicity of the *Alternaria* isolates on *Tupidanthus* and *Dizygotheca* and to document changes in conidial morphology under defined environmental conditions. A brief report has been published previously (12).

MATERIALS AND METHODS

Leaf spots and blights were collected from *D. elegantissima, T. calyptratus,* and *B. actinophylla.* These lesions were dissected from the leaves, washed in running tap water, surface-sterilized in 0.5% sodium hypochlorite for 5 sec, and placed on water agar. Numerous conidia were produced after 5–7 days and several single-conidial isolates were established for each of the three host species. Spores of isolates from all three hosts were similar, indicating that they all belonged to a single *Alternaria* species. Pathogenicity studies were limited to representative isolates from each of the three hosts: isolate 769 (ATCC 46321) from *Dizygotheca*, 770 (ATCC 46322) from *Tupidanthus*, and 768 (ATCC 46320) from *Brassaia*. All three isolates were determined to be *A. panax* (11).

Cultures were grown on vegetable juice agar (VJA = 10 ml of V-8 juice, 90 ml of deionized water, 0.2 g of $CaCO_3$, and 1.8 g of agar) at 24 C under continuous cool-white fluorescent illumination of about 2,700 lux. Colonies were 5 and 7 days old, respectively, for morphological and pathological studies. Conidial suspensions for inoculations were adjusted to 10^4 spores per milliliter in a 1:2,000 Tween 20 solution.

Dizygotheca, Tupidanthus, and *Brassaia* plants about 4–8 mo old were inoculated with each of the three isolates by spraying conidial suspensions onto leaves until runoff. Plants were incubated in moisture chambers for 24 hr at ambient laboratory temperatures (about 24 C). Plants were returned to the greenhouse and symptom development was observed periodically. Pathogenicity tests were performed three times.

Conidial measurements of *A. panax* isolates 768, 769, and 770 were made by suspending spores from 5-day-old cultures in a drop of aqueous 0.05% agarose solution to minimize Brownian movement. Spore body length and diameter of the spore at its widest part were measured. The spore body was distinguished from the beak by excluding any elongated cell in the transition area from the body. Twenty-five spores each, all more than 50 μm in total length and thus presumed to be the first spore in the chain, were measured. The second spore was distinctly smaller, usually less than 50 μm long, whereas subsequent spores were even smaller.

Conidia produced on infected *Brassaia* leaves and on VJA were compared to document suspected differences. Leaf spots of *B. actinophylla* caused by isolate 768 were surface-sterilized with 0.5% sodium hypochlorite, then placed in a moist chamber for 3 days at 24 C under continuous fluorescent irradiation. Conidia from the lesion surface were measured as described previously and compared with conidia from 5-day-old cultures of isolate 768 produced at 24 C with light on VJA.

To study the development of large, asymmetric conidia, isolate 768 was grown on autoclaved *Brassaia* petioles. Colonies developed with loosely spaced conidiophores on petioles, which enabled direct selection of conidia in contrast with densely packed conidiophores on VJA or *Brassaia* leaves. Cultures were grown for 5 days at 24 C under continuous cool-white fluorescent illumination (about 2,700 lux), then placed in the dark for 3 hr at 16 C for conidial induction (1). Cultures were then transferred to 16, 20,

Journal Series 2671 of Hawaii Institute of Tropical Agriculture and Human Resources.

Accepted for publication 27 January 1984.

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24, and 28 C in the dark to allow spore development. After 24 hr, solitary conidia and the basal conidium of spore chains were collected individually with a glass needle. At 20 C, cultures were maintained for another day to obtain 48-hr-old basal conidium of spore chains for measurement. Twenty-five conidia from each treatment were measured in each of two duplicate tests.

RESULTS AND DISCUSSION

Twenty-four hours after inoculation, numerous water-soaked flecks were evident on the abaxial leaf surfaces of *Dizygotheca* and *Brassaia* plants. Brown to olive green, circular, depressed spots, 0.5–3 mm in diameter with broad chlorotic zones, occurred on *Dizygotheca* and *Brassaia* leaves 2–3 days after inoculation. Larger spots were water-soaked and appeared primarily on the abaxial surface. By 7 days postinoculation, spots developed into circular to irregular-shaped blights that resembled field-collected disease specimens. On *Brassaia*, spots were characterized by brown centers with tan borders, occasionally with slight water-soaking. On *Dizygotheca*, spots were yellow-brown to orange-brown and slightly raised at the edges on the abaxial surface. On both hosts, rapidly expanding lesions were brownish black with diffuse borders. Multiple spots, single large spots, or infection of a leaflet-stalk led to defoliation. Infection of unexpanded leaves or young stems produced elongate dark brown stem lesions and killed stem apices. On mature, hardy leaves, spots were restricted to 5 mm or less in diameter and were depressed, tan, and had raised light tan corky borders. On *Tupidanthus*, spots were considerably smaller than on *Dizygotheca* or *Brassaia*, even 7 days after inoculation. Most spots were less than 1 mm in diameter, circular to irregular, brown to orange-brown, depressed in the center, and had dark green borders. Larger spots, more than 2 mm in diameter, were irregular in shape, dark brown to black, and had water-soaked margins surrounded by a faint chlorotic halo.

Pathogenicity of the three isolates on each of the three host species was quite similar. Disease development and symptomatology on *D. elegantissima* resembled that on *B. actinophylla*. The youngest three or four leaves of *D. elegantissima* were completely blighted, whereas small, restricted corky spots were characteristic on older leaves. In these studies, *T. calyptratus* was the least susceptible, *D. elegantissima* was moderately susceptible, and *B. actinophylla* was the most susceptible.

A. panax was reported to cause leaf spots of dwarf schefflera (*Schefflera arboricola* (Hayata) Merrill) (2) and Ming aralia (*Polyscias fruticosa* (L.) Harms) (3) in Florida. We have also observed the disease on dwarf schefflera in Hawaii. Lesions on *S. arboricola* were usually restricted and had a faint yellow halo. Isolates obtained from *S. arboricola* were pathogenic to *B. actinophylla* and were morphologically similar to isolate 768.

In culture, conidia of all seven isolates had similar dimensions and olivaceous brown to dark golden brown color and occurred singly or in chains of three or more. In addition, they were highly variable in shape but generally obclavate to obpyriform to ovoid, muriform, with deep constrictions at the septa (Fig. 1). The means and standard deviations of conidial dimensions for a typical isolate (768) grown on VJA were as follows: 71.2 ± 9.7 μm (total length); 50.7 ± 9.1 μm (body length); 24.5 ± 4.0 μm (diameter). These are comparable to measurements given by Atilano (2,3).

In contrast, Miller (9) reported conidia for Florida isolates to range from 70 to 185 μm long and 18 to 45 μm wide on unspecified substrates. Our isolate 768 produced conidia on infected *Brassaia* leaves which were significantly ($P<0.05$) longer and broader (105.2 ± 20.2 × 29.4 ± 3.9 μm), than conidia formed on VJA (72.5 ± 14.8 × 21.8 ± 4.8 μm). These data bridge the substantial differences between data of Miller (9) and Atilano (2,3).

Conidial morphology varied considerably with temperature of spore development; the longest conidia formed at 20 C and the widest at 28 C, the highest temperature in these studies. Generally, solitary conidia were longer and broader than the basal conidium of chains at all temperatures; at 20 C, they were 155.7 ± 17.5 × 28.4 ± 3.0 μm. On autoclaved petioles at 20 C, 24-hr-old basal conidia were slender and longitudinally symmetrical (136.4 ± 18.7 × 27.1 ± 2.3 μm) (Fig. 2). Basal conidia that were maintained for 48 hr at 20 C were longer and broader (173.4 ± 22.5 × 33.3 ± 4.3 μm) than 24-hr conidia, and were asymmetrical or severely distorted (Fig. 3).

In culture, conidia of *A. panax* also resembled conidia of *A. raphani* Groves & Skolko. However, *A. raphani* is distinguished by shorter conidiophores and the development of abundant chlamydospores on malt agar after 10 days at 20 and 24 C. Two isolates obtained from crucifers in Hawaii were not pathogenic to *B. actinophylla* but caused severe blighting on radish (*Raphanus sativus* L.). Isolates 768 and 769 were not pathogenic to radish. Miller (10) applied the name *A. actinophylla* to the causal agent of *Brassaia* leaf blight in Florida. Although it has found practical application (4,8), *A. actinophylla* is not a legitimate name because it meets few criteria for validity (11). This fungus should be referred to as *A. panax*.

In his studies of *Alternaria* species

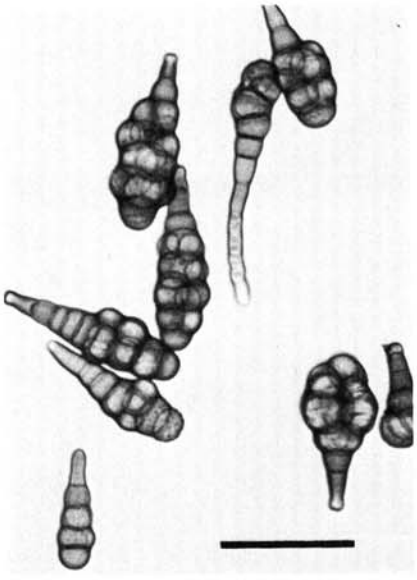

Fig. 1. Conidia of *Alternaria panax* formed at 24 C on V-8 juice agar. Bar = 50 μm.

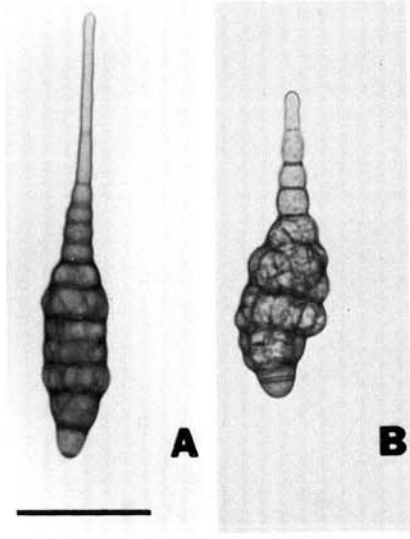

Fig. 2. Conidia of *Alternaria panax* formed on autoclaved petioles; induced for 24 hr at **(A)** 16 C and **(B)** 28 C. Bar = 50 μm.

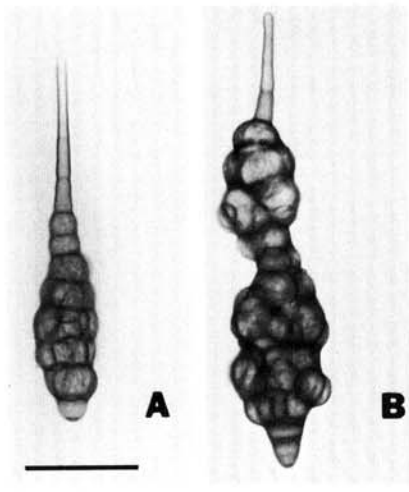

Fig. 3. Basal conidia of *Alternaria panax* formed at 20 C on autoclaved petioles; **(A)** 24-hr induction, **(B)** 48-hr induction. Bar = 50 μm.

from Araliaceae, Simmons (11) recognized 10 small-spored specimens as belonging to the *A. alternata-tenuissima* complex and declared that 25 remaining large-spored specimens were *A. panax*. Included among these 25 were Miller's (9) isolates and *Alternaria* specimens from *Aralia* spp., previously identified as *Macrosporium araliae* Dearness & House (5) and *A. araliae* Greene (6). Our isolates 768, 769, and 770 were also included among the isolates studied by Simmons (11).

Although Greene (6) was emphatic that *A. araliae* and *A. panax* were distinct species, Simmons (11) noted that Greene described specimens with freshly sporulating young lesions, in contrast to Whetzel's (13) description of specimens with "totally invaded stems" having fungal development of varied ages. This age difference may account for the contrasting conidial dimensions given for *A. araliae* and *A. panax*. Conidia closely fitting the description for *A. araliae* were obtained by Simmons (11) on freshly collected leaf spots. After incubation for 24 hr, these same lesions yielded conidia that were characteristic of *A. panax*. Our findings on changes in conidial morphology were similar to these.

Joly (7) placed *A. panax* within a group of *Alternaria*s that he rejected or considered of uncertain status. Simmons (11), however, proposed the retention of *A. panax* on the basis of Whetzel's (13) description, illustrations, availability of excellent type specimens, and the body of information associated with *A. panax*, and we concur.

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